REMARKS

Reconsideration of the patentability of the claims of the instant application is solicited in view of the above amendments and the following comments

In the outstanding action, the examiner has sought to support his rejection of the patentability of the claims of the instant application on the basis of the disclosure of the cited Nett et al. patent in combination with a variety of other references. It is pointed out, and has repeatedly been pointed out in past submissions, that the disclosure of the Nett et al. reference is of a conjugate that has been made by conventional chemical reaction techniques. This preparative procedure inserts at least one bridging moiety between the two portions of the instant claimed protein molecule. The instant claimed molecule is a single protein with no bridging moiety between the cell targeting portion of the protein and the cell killing portion of the protein.. Thus, the instant claims define the claimed protein as having been made by fusing at the cDNA level of the two moieties that make up the claimed protein.

GnRH-PE chimeric proteins claimed herein and the GnRH-toxin conjugates of the prior art represent different biological molecules that are not obvious from one another.

The GnRH-PE chimeric proteins of this invention are fusion molecules that have been assembled at the level of cDNA by genetic engineering techniques. The GnRH-PE chimeric proteins claimed herein are expressed and produced in bacterial expression systems. For practical application, the protein product is then preferably highly purified by a multi-step purification protocol. Each GnRH-PE chimeric protein claimed herein is a **single protein molecule** that has been fully characterized and found to be a highly purified and homogenous singular protein.

The GnRH-toxin conjugates of the prior art, on the other hand, are molecules in which the two moieties, the targeting moiety (GnRH) and the killing moiety (toxin) were **chemically conjugated using conventional chemistry (Nett et all) or by the**

conventional techniques of synthetic organic chemistry (Lombardo et al.). Since toxins commonly have multiple ligand attachment sites (generally amino groups or carboxy groups) and the conventional linkage chemistry is not selective with regard to the number of GnRH molecules activated or the places in the molecule where the molecule becomes bonded, there is no control of the degree of conjugation. That is, conjugation can occur at one or through multiple disparate attachment points. Thus, the final GnRH-toxin conjugate preparation made by prior art techniques is a **mix of products, possessing molecules with different ratios of ligand (GnRH)/toxin.**

Preparation of composite molecules by synthetic organic chemistry techniques lead to site-specific constructs. However, in both chemical techniques both the targeting moiety (GnRH) and the killing moiety (PE or any other toxin) undergoes chemical deviations/changes before chemical conjugation can occur. This leads to changes in their structure and functions, such as: affinities of binding, specificity in recognition, biological activity, etc. By any biological or chemical definition, GnRH-toxin chemical conjugates and GnRH-PE chimeric proteins are different molecules. The chimeric proteins of this invention differ in their sequence, structure (secondary and three dimensional structure), etc. from the chemical conjugate. Thus these chemical additive compounds will likely differ in their biological properties, such as: affinity, specificity, specific activity (meaning also efficacy), stability and more, as well.

In the outstanding action, the examiner has rejected the patentability of all of applicant's claims as lacking an innovative step (obvious under 35 USC 103) considering the combined disclosures of the cited Nett et all and Chaudhary et al. references. In general, targeted fused chimeric proteins, produced by genetic engineering techniques and comprising of a cell targeting moiety and a cell killing moiety, are well described and known in the literature for over 15 years. The use of PE-40 and the mutated full length PE (as claimed herein) is admittedly disclosed by the Chaudhary et al. reference. However, using a cell-targeting moiety of only 10 amino acids such as the Gonadotropin Releasing Hormone (GnRH) in the chimeric protein claimed herein is not an obvious target/sequence to be chosen. Moreover, to assume that a GnRH-toxin chimeric protein should work in the

IV PRIOR ART

same manner as any other typical/common chimeric protein is not a correct or an obvious expected result. Using different kinds of targeting moieties, a large number of chimeric proteins/immunotoxins have been generated in the last 20 years by chemical linkage techniques or recombinant DNA technology. The size of the targeting moieties has varied widely, ranging from large antibodies (of about 150KDa in size) to relatively small growth factors, cytokines and antibody fragments (of about 10-20KDa in size). **However, to the best of applicant's knowledge, a peptide of 10 amino acids (being only ~1KDa in size) has never before been proposed or used in the construction of a chimeric protein.** The ability of a chimeric protein like the GnRH-PE construct claimed herein to target cells via a very small peptide constituent (only 10 amino acids, having a molecular weight of about 1 KDa) fused to a much larger protein having a molecular weight of about 40KDa or 66KDa (such as the two forms of the PE toxin specifically described and claimed herein) and yet retain its original functions, namely specific binding and internalization into the target cells, offers new possibilities for designing targeted chimeric proteins and are highly innovative.

Thus, GnRH-PE chimeric proteins claimed herein represent an innovative approach for constructing a new sub-class of recombinant fusion chimeric proteins based on very small targeting sequences (10 to 30 amino acids) fused to a much larger protein (such as a toxin).

GnRH-toxin conjugates and GnRH-PE chimeric proteins recognize and target different receptors

In all patents/references cited by the examiner in support of his rejections of the patentability of applicant's claims, the GnRH-toxin chemical conjugates are targeting the pituitary gland, or more precisely the gonadotropin secreting cells of the anterior pituitary gland that are expressing the GnRH receptor. **The instant claimed protein construct is not targeting the known and cloned human pituitary-GnRH-receptor but rather a GnRH-binding-site that although not yet cloned, is different from the human**

pituitary-GnRH-receptor. This GnRH-binding site is expressed mainly on adenocarcinomas.

There is being filed herewith, and incorporated herein by reference, a declaration under the provisions of 37 CFR 1,132. This declaration submits evidentiary facts that support the distinction between the proteins claimed in this application and the molecules of the reference(s)

Using primary cultures established from human granulosa cell tumor, the applicant has demonstrated that these cells express the human pituitary GnRH receptors (known today as type I GnRH receptors) as proven by PCR (polymerase chain reaction) analysis (Please see Fig. 1 of the attached declaration). However, these cells are not killed and not affected by the GnRH-PE66 chimeric protein (Please see Table 1 of the attached declaration). As a control for the activity of the GnRH-Pe66 chimeric protein, the GnRH-PE66 construct of this invention was tested on target Caco2 colon adenocarcinoma cells that are efficiently being killed by the chimeric protein (Please see Table 1 of the attached declaration).

These results suggested that, most probably, GnRH-PE is not recognizing and not targeting the known human pituitary GnRH receptors, but rather is recognizing and targeting other, different, GnRH-binding sites that, as demonstrated by the attached data, are expressed on adenocarcinoma cells.

Therefore:

1. Mechanism of action of the molecules of this invention is different from the mechanism of action of the molecules of the prior art

The mechanism of action of the conventional, prior art GnRH-toxin conjugate for treatment of hormone-dependent types of cancers is completely different from the action of the chimeric protein of this invention. The GnRH-toxin conjugate of the prior art has been proposed to work not directly on the cancer cells but rather by an indirect effect; because

the conjugate is believed to " be specifically targeted to the gonadotropin-secreting cells of the anterior pituitary gland...the only cells to which the gonadotropin-releasing hormone portion of the conjugate will bind"... and thereby eliminate the gland's ability ..." to secrete LH and FSH and thus is rendered sterile.." Sex steroid-dependent tumors which respond to such hormonal manipulation are thus believed to have their growth controlled because of lack of steroid hormone secretions in a sterilized animal/human carrying such tumors.

To the contrary, the GnRH-PE chimeric protein of this invention works through a **direct action on the tumor cells. The tumor cells are killed** because, as supported by the attached evidence: adenocarcinomas express the GnRH binding sites; the claimed GnRH-PE chimeric proteins bind to these binding sites thus allowing the internalization of the instant chimeric protein directly into the adenocarcinoma cells. Upon internalization into the cancer cells, the Pseodomonas Exotoxin-killing moiety of the instant claimed construct inhibits protein synthesis (the natural activity of the toxin), thus leading to the death of the cells. Thus, GnRH-PE chimeric proteins claimed herein act directly by killing the cancer cells (adenocarcinomas).

Further:

2. Applications of the GnRH-toxin conjugates and GnRH-PE chimeric proteins molecules differ:

The GnRH-toxin conjugates of the prior art have been suggested mainly for the sterilization of animals (veterinary medicine). In human medicine, the reagents are suggested to be used for the following limited purposes; to control fertility (to achieve infertility effects), to treat sex steroid-dependent tumors, such as breast and prostate cancers (the only ones mentioned), and for the treatment of endometriosis. The applications for human medicine do not appear in the detailed claims of the Nett et al. reference.

As demonstrated by the results reported in the attached declaration, the instant claimed GnRH-PE chimeric protein is a cytotoxic agent for a wide verity of cancers. Most

surprisingly, the instant chimeric construct acts positively on cancers originated in non-hormone depended tissues, such as: colon carcinoma, lung carcinoma, renal carcinoma, hepatocarcinoma and more. Thus, the main applications of the instant claimed chimeric protein are against:

malignant carcinomas, including non-hormone dependent cancers (a wide verity of adenocarcinomas); and

benign tumors of the uterus and hyperplasia, including uterine lyomyoma, endometriosis, benign prostate hyperplasia, breast polycystic disease and pituitary adenoma.

GnRH-based chimeric proteins specifically target and kill only adenocarcinoma cells

More recently results reported in the accompanying declaration have verified the prior findings of the applicant that GnRH-based chimeric proteins very specifically target and kill only adenocarcinoma cells. A wide variety of killing protein moieties were used in the tested chimeric proteins (such as the PE toxin) or human apoptotic proteins (such as proteins of the Bcl-2 family-Bax, Bak, Bik, or the DNase DFF40), and all (when fused to the instant GnRH targeting moiety) caused cell death of only adenocarcinoma cells, irrespective of whether they were bacterial or human pro-apoptotic proteins. It should also be pointed out that the various killing moieties used to generate the data for the attached declaration not only differed in their origin but also in their size. These reported data further highlight the fact that the GnRH sequence is, indeed, responsible for targeting the various chimeric proteins to the target cells, and any protein fused to GnRH in the form of a chimeric protein will enter the cell via the GnRH-binding sites.

It should be clear that the instant claimed proteins are substantively different from the conjugates of the prior art. That they are substantively different is proven by the manner in which they operate and in the types of cancer cells that they address. It is Hornbook law that one must look at the properties of a claimed material, not only its name. The Court of Appeals for the Federal Circuit has made it abundantly clear that the

properties of a compound are inherent in the identification of that compound. The mere fact that the compound(s) of the prior art appear to have the same or a similar name does not support the fact that these prior art compounds are indeed the same as or obvious variants of the compounds being claimed herein. The mere fact that the starting materials superficially appear to be the same or similar to those used in the instant invention is of no moment.

The first most important thing is that the instant claimed proteins are absolutely new. In this regard, note that the examiner has only rejected the instant claims on an obviousness basis, not as being anticipated by the prior art. The second most important thing is that the instant claimed proteins behave differently from the closest prior art materials. This is an unusual and unexpected result and clearly supports the proposition that the instant claimed proteins are not at all obvious from a consideration of the state of the prior art as evidenced by the disclosures of the several references that have been cited by the examiner.

It is urged that the examiner carefully consider the evidence filed herewith, the specific language of the instant claims and the actual disclosures of the cited references. It is believed that such comparisons will support the finding that the instant claims define a patentable invention and should be allowed.

The examiner's objections/rejections under 35 USC 112 have been considered.

As to claim 1, the objected to language means that the GnRH portion of the claimed protein is adapted to bind to the cell binding site and therefore permits the killing portion of the instant protein molecule to enter the cell through that binding site and to thereby enable the killing moiety to kill the cell to which the protein is bound. The objected to phrase means that only the GnRH portion of the chimeric protein is recognizing and binding to the specific GnRH-binding sites, enabling the whole chimeric protein to be internalized into the target adenocarcinoma cell. It is clearly pointed out that the toxin is not binding the GnRH-binding site (**not by a covalent bond nor by any other bond**); the toxin's

substrate is intracellular and this portion of the chimeric protein is not involved et all in the recognition and binding of the chimeric protein to the GnRH-binding site that is expressed on the surface of the target adenocarcinoma cells. This mechanism is the basis for the whole action of targeting chimeric proteins. It is urged that the examiner reconsider this rejection and discontinue it.

As to the objection to the language of claim 29, it is indeed well known in the art that ATG (which codes for Met) is needed as the initiation codon for the protein to be produced and indeed the fusion cDNA sequence of this invention starts with ATG to allow translation of the chimeric protein. However, the issue as to whether the Met is processed off during the purification protocol does not lend itself to a straightforward or an obvious expected result. Some times the Met remains and some times Met is processed off, depending on each individual protein and purification protocol. In the case of GnRH-PE chimeric protein, the Met is not processed off during purification and the final chimeric protein product has a Met as its first amino acid. This was verified by an amino acids sequence analysis that was performed on the highly purified GnRH-PE chimeric protein (the official analysis is added). Fig. 1C shows that the Met was not processed off in that specific instance. (see page 8).

Allowance of all of the claims of the above referenced application is therefore solicited.

Respectfully submitted,

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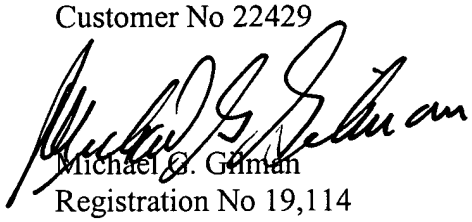

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